ORIGINAL RESEARCH

Queso Chihuahua: manufacturing procedures, composition, protein profiles, and microbiology

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A survey of fresh commercial Queso Chihuahua made from raw or pasteurized cow's milk was undertaken to determine the impact of cheesemaking parameters on composition, protein breakdown and microbial counts. Despite variations in the manufacturing procedure, the composition and casein breakdown of the cheeses fell within a comparatively tight range. Pathogens were not detected in any of the cheeses, but total aerobic plate counts exceeded the recommended limit for this variety. Differences in Mexican Queso Chihuahua manufacturing procedures did not substantially alter the final product, the quality of which could be improved by reduction of bacteria present.

Keywords Proteolysis, Queso Chihuahua, Raw milk.

INTRODUCTION

In 1922, some 20 000 Mennonites settled in the state of Chihuahua at the invitation of the Mexican president, who offered land and tax incentives if they would produce the bulk of cheese needed for northern Mexico (Macias and Torres 2000). Today, about 50 000 Mennonites live near the city of Cuauhtémoc, 105 km west of the capital of Chihuahua, where many are involved in small-scale (< 3000 kg/day) cheesemaking operations. Their specialty is known in Mexico as Queso Chihuahua or Chester cheese, a semihard minimally ripened product made from whole cow's milk (Van Hekken and Farkye 2003). A pasteurized version is made in the USA and is called Menonita or Chihuahua™ cheese. The manufacturing procedure for Queso Chihuahua resembles that of Cheddar (Saltijeral *et al*. 1999), but the product is consumed < 2 month after manufacture (Van Hekken and Farkye 2003). Although the literature describes this cheese as being manufactured from pasteurized milk (PM) (Phelan *et al*. 1993; Solano-López and Hernández-Sánchez 2000) or from pasteurized and homogenized milk (Saltijeral *et al*. 1999), much is actually made from nonhomogenized raw milk (RM) supplied by dairy co-operatives.

Fourteen per cent of Americans identify themselves as Hispanic or Latino (US Census Bureau 2005), which has created a demand for Hispanic-style cheeses (Van Hekken and Farkye 2003). US production of Hispanic-style cheese has climbed from < 31 000 000 kg in 1996 (US Department of Agriculture 1997), when tracking by the USDA began, to > 75 900 000 kg in 2005 (US Department of Agriculture 2006). However, the US Food and Drug Administration has not set standards of identity for Hispanic cheeses, and relatively little research on the properties of these varieties has been reported. The only papers in the literature about Queso Chihuahua deal with age-related changes in composition, microbial quality, and histamine and tyramine concentrations (Diaz-Cinco *et al*. 1992), and the possible presence of *Listeria monocytogenes* (Saltijeral *et al*. 1999; Solano-López and Hernández-Sánchez 2000). *Listeria* has been found in some RM samples and can survive the manufacture of Queso Chihuahua (Solano-López and Hernández-Sánchez 2000), but no outbreaks of listeriosis resulting from contamination of this variety have been reported and no *Listeria* was identified in a study of Queso Chihuahua (Saltijeral *et al*. 1999).

The Dairy Processing and Products Research Unit, ARS, USDA, in collaboration with Centro de Investigacion en Alimentacion y Desarrollo, is currently characterizing the chemical, physical, functional, rheological, and textural properties of Hispanic-style cheeses, including Queso Blanco and Queso Fresco. This effort includes the use of

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SDS-PAGE, which provides information on casein proteolysis in cheese (Strange *et al*. 1992), and studies of microflora, which is an area of concern with RM cheeses (Keene 1999). As part of this investigation, a number of Mexican Mennonite cheesemakers provided samples and information about manufacturing procedures with the goal of optimizing the quality of this variety. Variations with seasonality were covered in an earlier report (Tunick *et al*. 2007). This paper describes manufacturing parameters, composition, protein profiles, and microbiology of fresh (≤ 1 week old) Queso Chihuahua made in Mexico.

MATERIALS AND METHODS

Cheesemaking

Cheeses were made at local operations around Cuauhtémoc; the plants and the samples from them are designated by letters A to Q in this study. Cheeses A to K were made from RM. Cheeses L, M, and P were made from PM heated at 75°C for 15 s, and cheese N milk was pasteurized at 64°C for 30 min. Cheese Q milk was heat-treated at 73°C for 3 s; for this study, this was also considered to be a PM cheese. Cheeses A–D were obtained in August 2001 and June 2004; cheeses E–H in May 2001 and June 2004; cheeses J, K, M, and Q in March 2001 and June 2004; cheese L in September 2002, May 2003, and June 2004; cheese N in November 2001, September 2002, and May 2003; and cheese P in November 2001 and June 2004. Manufacturing information was obtained from in-plant interviews with the cheesemakers. A few of the parameters such as temperature of cheddaring (the step where curd slabs are stacked and flipped) were not measured in some of the plants, and three manufacturers were reluctant to provide some information due to proprietary concerns. Details of the manufacturing procedures varied among plants, but all followed the steps shown in Figure 1. Triplicate blocks of shrink-wrapped cheeses, a maximum of four brands per shipment, were sent in refrigerated coolers overnight to the Dairy Processing and Products Research Unit, ARS, USDA in Wyndmoor, Pennsylvania, for the analyses, which were performed within 1 week of cheese manufacture.

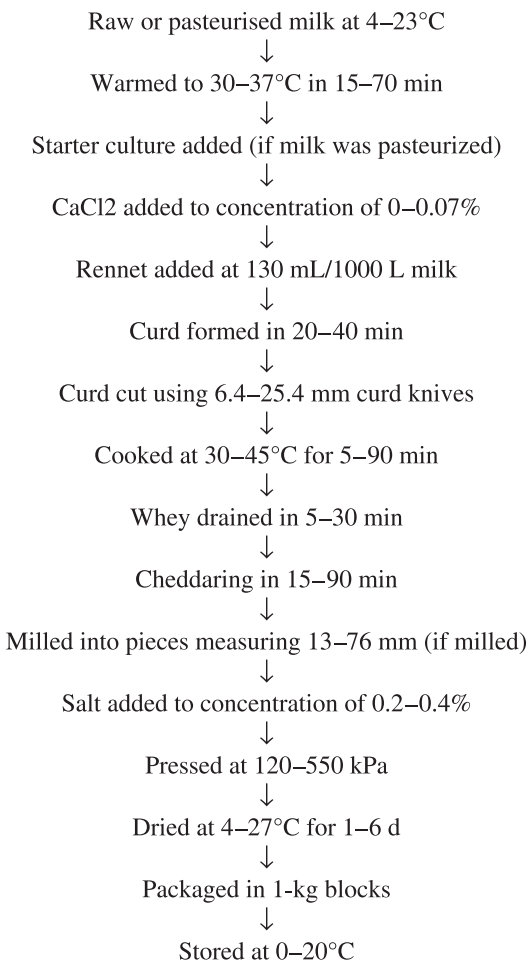

Figure 1 Manufacturing steps for Queso Chihuahua.

Composition

Moisture content was determined in triplicate by the forced-draft oven method 948.12 of AOAC International (2000), and fat content was determined in duplicate by the modified Babcock method (Kosikowski and Mistry 1997). Protein content was determined in duplicate by using an FP-2000 nitrogen analyser (LECO Corp., St. Joseph, MI, USA) and multiplying the percentage of nitrogen by 6.38. NaCl content was determined by high-range chloride titrators (Hach Co., Loveland, CO, USA). A model 611 pH meter (Orion Research Corp., Cambridge, MA, USA) equipped with a model 91-63 spear tip combination electrode (Orion) was used to measure pH.

Protein characterization

Cheese samples were frozen at –35°C 10 days after manufacture. After thawing at room temperature for 30 min, proteins were extracted using a modification of the procedure of Basch *et al*. (1989). Grated samples weighing 4 g were solubilized in 10 ml of pH 8 buffer (0.166 M Tris-HCl/1 mM EDTA) by homogenization for 10 min at 10 600 r.p.m. with a VirTis Tempest IQ^2 blender (VirTis, Gardiner, NY, USA). Ten millilitres of 7% SDS was added and the mixture was homogenized for 5 min at 8400 r.p.m. For protein reduction, the homogenate was transferred to a 50-ml polycarbonate centrifuge tube and 4 ml of freshly made 10 mM dithiothreitol (77 mg in 50 ml of buffer) was added. The tube was then stirred for 15 min in a 0°C bath and centrifuged for 1 h at 39 000 × *g* at 4°C in a Sorvall RC-5B centrifuge (DuPont Instruments, Wilmington, DE, USA). The supernate was filtered through laboratory wipes and lyophilized.

SDS-PAGE was run on 20% homogeneous gels using the PhastSystem (American Pharmacia Corp., Piscataway, NJ, USA). One microlitre of supernate, containing 5–10 μg of protein and protein fragments, was applied to each lane. After Coomassie Blue staining and destaining, the gels were scanned into a model 375A Molecular Dynamics Personal Densitometer SI equipped with ImageQuant software (Molecular Dynamics, Sunnyvale, CA, USA). Each lane was analysed in duplicate. Cow milk was used as a reference to identify major caseins (α_{S1}-CN, α_{S2}-CN, β-CN, and κ-CN) and whey proteins. Amounts of proteins and casein fragments were expressed as percentages of the combined total of intact proteins and peptides.

Microbiology

The outside surface of each cheese package was wiped with a paper towel soaked in 70% (w/v) ethanol. The packages were then opened and approximately 200 g was aseptically removed from the surface and anterior of each cheese using an ethanol-sterilized knife. Aerobic bacteria plate counts were determined following standard microbiological procedures for dairy products (Marshall 1992). Briefly, a 10-g portion from each cheese was transferred to a sterile nylon/polyethylene bag (3 milk STD Barrier; 20.3 × 30.5 cm; Koch Industries, Kansas City, MO, USA) containing 90 ml of sterile 0.1% (w/v) peptone water (Difco, Detroit, MI, USA) and macerated for 2 min. Each macerated cheese sample was serially diluted with 0.1% peptone water, and 50 μL of each dilution was direct plated onto duplicate nutrient agar plates (Difco) using a model 400 spiral plater (Spiral Biotech, Gaithersburg, MD, USA). Plates were manually counted and bacterial numbers were expressed as $\log_{10}$ colony-forming units per gram (cfu/g). The remaining portion of each cheese sample (about 190 g) was vacuum sealed to 950 mbar using a Multivac A300/16 vacuum-packaging unit (Sepp Haggemuller KG, Wolfertschwenden, Germany) in sterile nylon/polyethylene bags and shipped on ice to a commercial laboratory for additional microbiological analyses. Twenty-five-gram portions from each cheese sample were evaluated for the presence of *L. monocytogenes* (method 999.06) and *Escherichia coli* O157:H7 (method 999.10), using AOAC International (2000) procedures. In addition, the presence of *Staphylococcus aureus* enterotoxin and *Campylobacter* spp. were evaluated in 25-g portions using the Vidas immunoassay system (Bio Merieux, St. Louis, MO, USA).

Statistics

Statistics, including Pearson correlation coefficients, for relating manufacturing parameters, compositional parameters, proteins and peptides, and microbial counts to each other were obtained by using the SAS Software System (SAS 1999). A correlation is described as significant if $P < 0.05$.

RESULTS AND DISCUSSION

Manufacturing procedure

An understanding of the manufacturing procedure of Queso Chihuahua is crucial in evaluating the parameters that may be used to formulate standards of identity and standard manufacturing practices. The preparation of this cheese is patterned after Cheddar (Figure 1), but most Queso Chihuahua is made from RM, which contains indigenous microflora that eliminate the need for a starter culture. Table 1 shows the important manufacturing variables in Queso Chihuahua cheesemaking. In all but plant F, the curd was cut 30–40 min after addition of coagulant. Cheddar is usually cut into cubes approximately 6 mm on a side, but cubes up to 25 mm are used for Queso Chihuahua. The curd cooking temperature was higher than the 37–39°C range generally used for Cheddar in four plants, and was lower than that range in plants C and D. Curd cooking time is typically 45–60 min for Cheddar, but often only 5–30 min is required for RM cheese because of the variety and numbers of micro-organisms present. Four of the five PM cheeses were cooked for 45–90 min. Of the 10 plants where cheddaring temperature was reported, seven used elevated temperatures. Plant C (where the curd was cooked for 90 min), plant K (where curd cooking was 60 min), and plant G (where cheddaring lasted 90 min) cheddared the curd at ambient temperature. All plants except for G cheddared their cheese for 15–40 min. There was a wide variety in the milling procedure, where the curd is chopped into small pieces prior to NaCl addition. Curd milling size ranged from 13 to 76 mm, and plants J and Q did not mill at all. Between 1 and 4 g NaCl was added per kilogram of curd. Pressing parameters were also in a wide range. Plant N used a high pressure but the others were pressed between 120 and 280 Pa. The RM cheeses were pressed an average of 17.7 h, compared with 9.4 h for the PM cheeses.

The differences in cheesemaking procedures were due to availability of equipment and personal preferences. Empirical knowledge built up through trial and error allowed these manufacturers of Queso Chihuahua to manufacture a product that met their own standards.

Appearance and composition

The cheeses consisted of firmly packed curds with minimal air pockets between major curd divisions. All cheese blocks were pale yellow and some brands had a mottled appearance. A few of the RM cheeses displayed pinhole gas formations because of microbial fermentation.

Table 1 Key manufacturing variables of Queso Chihuahua cheeses made from raw milk (samples A through K) and pasteurized milk (samples L through Q)

	Cutting		*Cooking*		*Cheddaring*				*Pressing*	
Sample	*Coagulation time (min)*	*Cube size (mm)*	*Temperature (°C)*	*Time (min)*	*Temperature (°C)*	*Time (min)*	*Milling size (mm)*	*NaCl added (g/kg)*	*Pressure (Pa)*	*Time (hr)*
A	30–35	7	39	15	38	30	13–50	4.0	140	18
B	30	7	39	8	ND[a]	20	25	3.0	ND	20
C	40	19	32	90	23	20	19	1.1	160	16
D	ND	19	30	15	ND	20	64	3.0	240	17
E	30	6	38	45	38	20	13	4.0	170	18
F	20–30	13	45	5	30	15	51	3.0	280	14
G	30	13	40	20	22	90	13–76	3.6	210	24
H	30–40	6	39	30	39	40	13–19	3.0	140	18
J	30	13	38	45	34	20	none[b]	2.5	190	12
K	40	6	40	60	19	20	13	1.0	120	20
L	30	25	39	15	ND	ND	51	ND	ND	6
M	30	25	39	60	ND	ND	51	ND	ND	6
N	40	13	37	45	36	40	ND	4.0	550	15
P	30	ND	43	90	ND	ND	ND	3.0	280	8
Q	30	13	39	45	34	20	none	2.5	190	12

[a]ND, no data or insufficient data. [b]Curd not milled.

Table 2 Composition and aerobic bacterial counts of Queso Chihuahua cheeses made from raw milk (samples A through K) and pasteurized milk (samples L through Q)[a]

Sample	*Moisture (%)*	*Fat (%)*	*Protein (%)*	*pH*	*Aerobes $\log_{10}$ (cfu/g)*
A	39.4	34.3	24.1	4.82	8.78
B	39.7	31.9	25.1	4.98	8.92
C	41.6	32.5	24.4	4.80	8.97
D	39.5	31.9	26.7	4.89	9.35
E	38.8	32.4	27.3	5.22	9.61
F	38.3	34.1	27.6	4.93	8.82
G	39.8	32.5	27.2	5.24	9.41
H	41.3	30.2	26.0	4.92	9.01
J	36.5	33.0	25.1	5.25	7.47
K	39.0	33.2	25.1	5.00	8.53
L	41.1	32.1	23.8	4.82	6.08
M	42.4	31.3	25.5	5.25	6.82
N	41.7	32.1	25.1	5.26	8.07
P	44.4	31.8	24.7	5.21	7.35
Q	40.8	30.1	27.0	5.21	8.76
A-K mean[b]	39.4 ± 1.4	32.6 ± 1.2	25.9 ± 1.3	5.01 ± 0.17	9.12
L-Q mean	42.1 ± 1.4	31.5 ± 0.8	25.2 ± 1.2	5.15 ± 0.19	8.16
Mexican std.[c]	≤ 45.0	≥ 25.0	≥ 22.0	5.5–6.0	5.70
Cheddar[d]	37.0	32.0	25.0	5.50	6–8

[a]Means of three cheese blocks, each analysed in duplicate. [b]Mean of these samples ± standard deviation. [c]Mexican official standard (Saltijeral *et al.* 1999). [d]Data for typical American Cheddar cheese (Fox *et al.* 2000).

Despite the wide variation in the details of the manufacturing procedures, the cheese composition fell into a relatively narrow range (Table 2), an indication that Queso Chihuahua cheesemakers are able to obtain similar products while using procedures that they prefer. There were no significant correlations between manufacturing and compositional parameters. The mean moisture content of the RM cheeses was lower than that of the PM cheeses, and the fat and protein contents were correspondingly higher. The mean pH of the RM cheeses was also lower than that of the PM cheeses. Overall, the cheeses contained 40.3 ± 1.9% moisture (ranging from 36.5 to 44.4%), 32.2 ± 1.2% fat (30.1–34.3%), and 25.6 ± 1.2% protein (23.8–27.6%). The NaCl content of the cheeses was

between 1.0 and 1.5%. All cheeses in this study complied with the Mexican official standards for this variety (Solano-López and Hernández-Sánchez 2000), except the pH values were low. The compositional data reported here are similar to data reported elsewhere. The National Nutrient Database published by USDA (2004) lists Queso Chihuahua as containing 39.1% moisture, 29.7% fat, and 21.6% protein. Diaz-Cinco *et al*. (1992) found that 8-day-old cheeses from the states of Chihuahua and Sonora contained 41.9% moisture, 21.2% fat, and 26.4% protein. Queso Chihuahua that was manufactured by Solano-López and Hernández-Sánchez (2000) contained 36.4% moisture, 31.9% fat, and 24.9% protein, which is similar to the composition of Cheddar (Table 2).

Protein profiles

Profiles representative of the proteins extracted from the cheeses are shown in Figure 2. The profiles of the RM cheeses (lane 2 and 3) and the PM cheeses (lane 4 and 5) were typical of fresh cheeses, including the presence of the major proteins and the major peptides. Chymosin, the principal proteinase in rennet, coagulates milk by hydrolysing κ-CN into κ-CN (f1–105), also called para-κ-CN, and κ-CN (f106–169), which is lost in the whey. Chymosin also cleaves α_{s1}-CN into α_{s1}-CN (f1–23), which is rapidly degraded, and the α_{s1}-CN (f24–199) peptide, commonly abbreviated α_{s1}-I-CN (Mulvihill and Fox 1979). This peptide has been used to monitor proteolysis during cheese ripening (Tunick *et al*. 1993). The percentages of the various caseins, major peptides, and casein fragments are shown in Table 3. Variations within the RM cheeses and the PM cheeses may have resulted from differences in manufacturing technique; for instance, the relatively high level of whey proteins in cheeses from plant A could have been due to less vigorous removal of whey from the curd. The 90 min of cheddaring in plant G (no other plant cheddared more than 40 min) was probably responsible for the low amount of α_{s1}-CN and the elevated levels of casein fragments in their cheese.

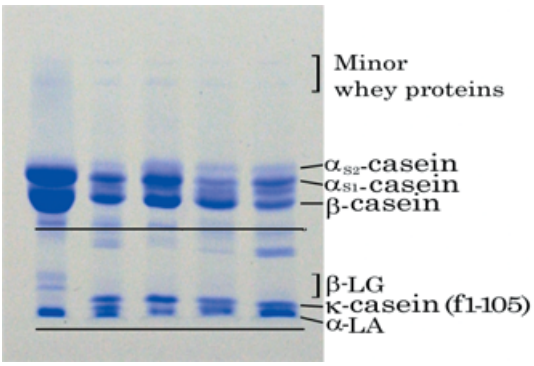

Figure 2 Proteins and casein fragments in Queso Chihuahua as viewed by PAGE. Lane 1 is a cow milk standard, lanes 2 and 3 are cheeses made from raw milk, and lanes 4 and 5 are cheeses made from pasteurized milk. Upper and lower horizontal lines correspond to 20 and 12 kDa, respectively.

There was a relationship (Pearson correlation coefficient $r = -0.832$) between α_{s1}-CN degradation and α_{s1}-CN (f24–199) formation. Chymosin cleaves α_{s1}-CN (f24–199) to yield a number of peptides, especially α_{s1}-CN (f33–199), α_{s1}-CN (f102–199), and α_{s1}-CN (f110–199) (Banks 2003), with molecular weights of 19.8, 11.1, and 10.7 kDa, respectively (E.L. Malin, 2005, personal communication). Plasmin, an indigenous milk protease associated with casein micelles and found in cheese (Farkye 1995), is not deactivated by pasteurization and is almost entirely retained in Cheddar curd at concentrations in the 3.0–4.5 μg/g range (Banks 2003). Plasmin hydrolyses β-CN to form peptides such as β-CN (f29–209), β-CN (f106–209), and β-CN (f108–209) (Sousa *et al*. 2001), with molecular weights of 20.5, 11.8, and 11.6 kDa, respectively; the complimentary peptides have molecular weights under 13 kDa (E.L. Malin, 2005, personal communication). Plasmin also attacks α_{s1}-CN (f24–199) to yield α_{s1}-CN (f104–199), α_{s1}-CN (f106–199), and other peptides (Banks 2003). Intact α_{s1}- and β-CN displayed inverse relationships to the percentages of 18.5–22 kDa and 10–14 kDa casein fragments in the Queso Chihuahua (Table 4), suggesting that chymosin cleavage of α_{s1}-CN (f24–199) and plasmin cleavage of β-CN occurred. Moreover, the combined amount of α_{s1}- and β-CN displayed significant correlations with the percentages of 18.5–22 kDa and 10–14 kDa casein fragments. Further study would identify which enzyme source was responsible for specific peptide formation.

The percentages of α_{s1}-CN (f24–199), 18.5–22 kDa casein fragments, and 10–14 kDa casein fragments were slightly and insignificantly higher in the RM cheeses, suggesting that microflora and indigenous enzymes in the RM might have enhanced proteolysis to a small extent. This effect becomes more pronounced when the cheeses are aged longer, as observed in Cheddar cheese (Marth 1963) and in Queso Chihuahua stored for 16 weeks (Lau *et al*. 1991).

Microbiology

Every block of cheese tested negative (< 0.04 cfu/g or 0.04 mg protein) for *L. monocytogenes*, *E. coli* O157:H7, *Campylobacter* spp., and *S. aureus* enterotoxin. Diaz-Cinco *et al*. (1992) evaluated five cheeses from Chihuahua and Hermosilla and were not able to detect the presence of *L. monocytogenes*, *Yersinia enterocolitica, Salmonella,* or *Shigella.* Saltijeral *et al*. (1999) tested 40 cheeses from Mexico City and did not detect the presence of *L. monocytogenes.* Solano-López and Hernández-Sánchez (2000) added *L. monocytogenes* to cheese

Table 3 Percentages of proteins and casein fragments in Queso Chihuahua cheeses made from raw milk (samples A through K) and pasteurized milk (samples L through Q)[a]

Sample	Whey proteins	Caseins and major peptides					Casein fragments (kDa)		
		α_{s2}	α_{s1}	α_{s1} (f24–199)	β	κ(f1–105)	18.5–22	14–18	10–14
A	3.09	6.14	26.26	2.19	24.91	10.12	14.58	4.24	8.48
B	0.92	7.77	25.84	4.95	28.78	11.55	10.28	3.52	6.40
C	1.48	7.73	26.37	4.42	31.44	11.38	8.80	2.97	5.44
D	1.43	7.48	26.25	4.18	29.09	11.34	9.94	4.12	6.19
E	0.92	7.48	26.80	3.68	29.50	12.43	9.89	2.91	6.40
F	1.14	6.60	21.62	6.67	29.50	12.87	11.42	2.78	7.41
G	1.25	4.80	17.59	5.18	23.99	12.24	17.20	3.57	14.19
H	0.97	6.33	23.26	5.48	26.48	12.70	14.98	1.12	8.68
J	0.48	7.44	23.10	5.35	32.42	11.14	10.17	2.09	7.84
K	1.53	7.10	26.20	4.96	29.98	12.04	8.91	2.46	6.84
L	0.81	9.29	22.80	4.56	31.27	14.50	7.93	3.93	4.94
M	1.32	7.24	18.67	8.26	29.74	12.21	11.64	3.37	7.57
N	1.06	8.00	26.08	4.05	24.64	11.05	12.53	3.55	8.92
P	2.07	6.87	33.31	0.00	33.18	10.96	6.30	2.61	4.72
Q	0.93	8.20	23.74	5.91	29.38	13.23	9.92	3.47	5.25
A–K mean[b]	1.32 ± 0.70	6.89 ± 0.93	24.33 ± 2.96	4.71 ± 1.20	28.61 ± 2.70	11.78 ± 2.70	11.62 ± 2.91	2.98 ± 0.95	7.79 ± 2.48
L–Q mean	1.24 ± 0.50	7.92 ± 0.94	24.92 ± 5.40	4.56 ± 3.02	29.64 ± 3.17	12.39 ± 1.50	9.67 ± 2.57	3.39 ± 0.48	6.28 ± 1.87

[a]Means of three cheese blocks, each analysed in duplicate. [b]Mean of these samples ± standard deviation.

Table 4 Pearson correlation coefficients for caseins and casein fragments in Queso Chihuahua cheeses[a]

Proteolysis product	α_{s1}-*CN*	β-*CN*	α_{s1}- + β-*CN*
α_{s1}-CN (f24–199)	–0.832		
18.5–22 kDa	–0.605	–0.885	–0.874
14–18 kDa	–0.063	–0.261	–0.178
10–14 kDa	–0.602	–0.764	–0.810

[a]Means of three cheese blocks, each analysed in duplicate.

milk prior to making Queso Chihuahua and found that *L. monocytogenes* survived processing and remained viable up to 6 weeks in the cheese.

Aerobic microbial counts ranged from 8.53 to 9.61 $\log_{10}$ cfu/g for all of the RM cheeses except for J, which contained 7.47 $\log_{10}$ cfu/g (Table 2). The range for the PM cheeses, 6.08–8.76 $\log_{10}$ cfu/g, was broader. The standard recommended by the Mexican government is 5.70 $\log_{10}$ cfu/g (Diaz-Cinco *et al*. 1992). High bacterial levels enhance proteolysis, which degrades the protein matrix, creates off-flavours and limits shelf life, a consideration if these cheeses are to be widely marketed. Microbial growth in this variety is relatively large because of the indigenous microflora (both in the milk and in the air at the cheese plant), elevated moisture, and reduced salt relative to Cheddar. The total counts are similar to the 8.53 $\log_{10}$ cfu/g reported by Diaz-Cinco *et al*. (1992) after 12 days of refrigerated storage. Bricker *et al*. (2005) found that mesophilic and thermophilic lactococci were present at 6.41–8.75 $\log_{10}$ cfu/g in both PM and RM Queso Chihuahua. RM cheeses contained 7.14–8.93 $\log_{10}$ cfu/g *Leuconostoc* ssp., mesophilic *Lactobacilli*, and non-*Lactobacillus* mesophiles, whereas PM cheeses contained 3.42–7.24 $\log_{10}$ cfu/g.

RM Cheddar cheeses have long been known to contain elevated aerobic counts when compared to PM cheeses that were inoculated with commercial starter cultures (Tunick *et al*. 2007). Claims of superior cheese flavour due to the use of RM are dependent on indigenous micro-organisms present in the milk when it arrives at the cheese plant. There were no significant correlations between microbial counts and protein profiles in the cheeses in this study, indicating that the number and strains of micro-organisms affected greatly proteolytic breakdown of casein to a small extent, if at all. The fact that PM Queso Chihuahua is being sold successfully indicates that the elevated microbial levels in the RM cheeses are not necessary to achieve an acceptable product with a characteristic flavour (Bricker *et al*. 2005). Further investigation into the microflora present in Queso Chihuahua is establishing levels and types of micro-organisms present (Bricker *et al*. 2005) and should enhance our insight into the flavour and textural differences noted between RM and PM cheeses.

SUMMARY

Queso Chihuahua cheeses were studied to provide information on manufacturing procedure, composition, proteolysis, and microbiology. The variety of manufacturing procedures used did not appreciably affect the composition of the product. With few exceptions, the composition, proteolysis products, and aerobic microbial counts of the RM Queso Chihuahua samples were similar. The composition and proteolysis products of the PM cheeses were also similar to each other, although the bacterial counts were wider ranging. RM cheese displayed higher moisture and bacterial counts than PM cheese. The pattern of early casein proteolysis indicated that chymosin and plasmin cleavage occurred, but did not significantly differ with pasteurization. Several types of foodborne pathogens were not detected, but total aerobic plate counts were above 6 $\log_{10}$ cfu/g for the cheeses, which appreciably increases proteolysis in the product. This information should provide guidance to cheesemakers wishing to improve the quality of this product.

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REFERENCES

Association of Official Analytical Chemists International (AOAC) (2000) *Official Methods of Analysis*, 17th edn. Gaithersburg, MD: AOAC International.

Banks J M (2003) Cheddar-type cheeses. In *Encyclopedia of Dairy Sciences*, Vol. 1, pp 356–363. Roginski H, Fuquay J W and Fox P F, eds. London: Academic Press.

Basch J J, Farrell H M Jr, Walsh R A, Konstance R P and Kumosinski T F (1989) Development of a quantitative model for enzyme-catalyzed, time-dependent changes in protein composition of Cheddar cheese during storage. *Journal of Dairy Science* **72** 591–603.

Bricker A L, Van Hekken D L, Guerrero V M and Gardea A A (2005) Microflora isolated from Mexican Mennonite-style cheeses. *Food Protection Trends* **25** 29–32.

Diaz-Cinco M E, Fraijo O, Grajeda P, Lozano-Taylor J and Gonzalez de Mejía E (1992) Microbial and chemical analysis of Chihuahua cheese and relationship to histamine and tyramine. *Journal of Food Science* **57** 365.

Farkye N Y (1995) Contribution of milk-clotting enzymes and plasmin to cheese ripening. In *Chemistry of Structure-Function Relationships in Cheese*, pp 195–207. Malin E L and Tunick M H, eds. New York: Plenum Press.

Fox P F, Guinee T P, Cogan T M and McSweeney P L H (2000) In *Fundamentals of Cheese Science*, pp 226 and 428. Gaithersburg, MD: Aspen Publishers.

Keene W E (1999) Lessons from investigations of foodborne disease outbreaks. *Journal of the American Medical Association* **281** 1845–1847.

Kosikowski F V and Mistry V V (1997) *Cheese and Fermented Milk Foods*, 3rd edn, Vol. 2, pp 212–214. Westport, CT: F.V. Kosikowski LLC.

Lau K Y, Barbano D M and Rasmussen R R (1991) Influence of pasteurization on protein breakdown in Cheddar cheese during aging. *Journal of Dairy Science* **74** 727–740.

Macias M and Torres F (2000) Mennonite colonies in Mexico accept change slowly. *Borderlands* **19** 13.

Marshall R T (1992) *Standard Methods for the Examination of Dairy Products*, 16th edn, pp 299–303. Washington, DC: American Public Health Association, Inc.

Marth Eh (1963) Microbiological and chemical aspects of Cheddar cheese ripening: a review. *Journal of Dairy Science* **46** 869–890.

Mulvihill D M and Fox P F (1979) Proteolytic specificity of chymosin on bovine α_{s1}-casein. *Journal of Dairy Research* **46** 641–651.

Phelan J A, Renaud J and Fox P F (1993) *Cheese: Chemistry, Physics and Microbiology. Major Cheese Groups*, Vol. 2, p 460. London: Chapman & Hall.

Saltijeral J A, Alvarez V B and Garcia B (1999) Presence of *Listeria* in Mexican cheeses. *Journal of Food Safety* **19** 241–247.

SAS Institute (1999) *SAS User's Guide: Statistics*. Version 8 Edition. Cary, NC: SAS Institute, Inc.

Solano-López C and Hernández-Sánchez H (2000) Behaviour of *Listeria monocytogenes* during the manufacture and ripening of Manchego and Chihuahua Mexican cheeses. *International Journal of Food Microbiology* **62** 149–153.

Sousa M J, Ardö Y and McSweeney P L H (2001) Advances in the study of proteolysis during cheese ripening. *International Dairy Journal* **11** 327–345.

Strange E D, Malin E L, Van Hekken D L and Basch J J (1992) Chromatographic and electrophoretic methods used for analysis of milk proteins. *Journal of Chromatography* **624** 81–102.

Tunick M H, Mackey K L, Shieh J J, Smith P W, Cooke P and Malin E L (1993) Rheology and microstructure of low-fat Mozzarella cheese. *International Dairy Journal* **3** 649–662.

Tunick M H, Van Hekken D L, Call J, Molina-Corral F J and Gardea A A (2007) Queso Chihuahua: Effects of seasonality of cheesemilk on rheology. *International Journal of Dairy Technology* **60** 13–21.

US Census Bureau Population Division (2005) *Annual Estimates of the Population by Sex, Race and Hispanic or Latino Origin for the United States: April 1, 2000 to July 1, 2004* (NC-EST2004-03). Washington, DC: US Census Bureau.

US Department of Agriculture, Agricultural Research Service (2004) *USDA National Nutrient Database for Standard Reference*, Release 17. Nutrient Data Laboratory Home Page, http://www.nal.usda.gov/fnic/foodcomp.

US Department of Agriculture, National Agricultural Statistics Service (1997) *Dairy Products 1996 Summary*. Washington, DC: USDA.

US Department of Agriculture, National Agricultural Statistics Service (2006) *Dairy Products 2005 Summary*. Washington, DC: USDA.

Van Hekken D L and Farkye N Y (2003) Hispanic cheeses: the quest for queso. *Food Technology* **57** 32–38.

